

# A comparison of methods to estimate nutritional requirements from experimental data

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**Abstract** 1. Research papers use a variety of methods for evaluating experiments designed to determine nutritional requirements of poultry. Growth trials result in a set of ordered pairs of data. Often, point-by-point comparisons are made between treatments using analysis of variance. This approach ignores that response variables (body weight, feed efficiency, bone ash, etc.) are continuous rather than discrete. Point-by-point analyses harvest much less than the total amount of information from the data. Regression models are more effective at gleaning information from data, but the concept of "requirements" is poorly defined by many regression models.

2. Response data from a study of the lysine requirements of young broilers was used to compare methods of determining requirements. In this study, multiple range tests were compared with quadratic polynomials (QP), broken line models with linear (BLL) or quadratic (BLQ) ascending portions, the

saturation kinetics model (SK) a logistic model (LM) and a compartmental (CM) model.

3. The sum of total residuals squared was used to compare the models. The SK and LM were the best fit models, followed by the CM, BLL, BLQ, and QP models. A plot of the residuals *versus* nutrient intake showed clearly that the BLQ and SK models fitted the data best in the important region where the

ascending portion meets the plateau.

4. The BLQ model clearly defines the technical concept of nutritional requirements as typically defined by nutritionists. However, the SK, LM and CM models better depict the relationship typically defined by economists as the "law of diminishing marginal productivity". The SK model was used to demonstrate how the law of diminishing marginal productivity can be applied to poultry nutrition, and how the "most economical feeding level" may replace the concept of "requirements".

#### INTRODUCTION

The problems in determining the best levels of essential nutrients in feeds were detailed by Lerman and Bie (1975). They pointed out two major obstacles to achieving diets that maximise profits. First, it is impractical to determine the nutritional content of every batch of raw ingredients. Therefore, costly margins of safety must be included in feed formulations to assure that minimum nutrient specifications (or "requirements") are met. Second, it is difficult to select realistic curves that relate animal response to

feed composition. Lerman and Bie (1975) demonstrated the importance of choosing the correct response model in achieving maximum profit formulations.

Animal feeding trials result in ordered pairs of data (Figure 1). Many different curves that relate animal response to feed composition can be fitted to data from animal feeding trials. The most realistic curve for each set of data is difficult to choose because of the variability in the responses of different individuals, or pens of individuals, fed on the same diet. The data points appear to be scattered about or around

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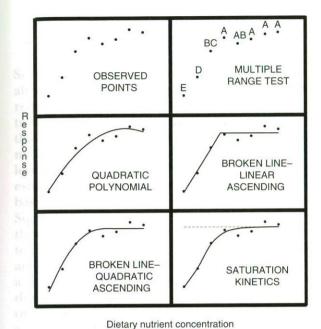


Figure 1. Several representations of responses to dietary nutrient level.

#### Kaleen.

a continuum with an ascending portion where the nutrient in question is limiting some response, such as growth, carcase composition, or lean meat yield. When enough of the nutrient is fed, a "plateau" results, where maximum (or minimum) performance levels have been achieved. If excessive concentrations of any nutrient are fed, the diet becomes imbalanced or the nutrient is toxic, resulting in decreased performance (not depicted in Figure 1).

The simplest, although misguided, analysis of nutritional response data is just to compare points using paired t-tests, orthogonal contrasts, or multiple-range tests. The requirement is defined as the lowest concentration of the nutrient that results in a response that is not significantly different from the maximum (or minimum) response at some arbitrarily chosen level of significance. Such interpretations always result in conclusions that the requirement is between two concentrations of the nutrient that were fed in the experiment. There is no way to tell exactly what the requirement is, or to tell the level of confidence in the requirement estimate. Since there is no function defined, interpolation between two known points is not even possible.

Another simple way to analyse nutrient response data is to fit a polynomial model or expression, usually a quadratic, to the data. With quadratic models, the requirement is defined as the nutrient concentration resulting in the maximum predicted response value. With quadratic polynomials, the ascending portion is curved, increasing at a decreasing rate until the

maximum is reached. There is a single maximum point, not a plateau, and further increases in nutrient concentration result in (predicted) reduced performance levels.

Many authors consider the ascending portion of the response and the plateau both to be straight lines (the Broken-Line Linear Model, or BLL Model). A straight line is usually a reasonable approximation of the ascending portion, although it is never possible to tell if there is a sharp break between the lines or a smooth transition. The intersection of the ascending line and the plateau gives an estimate of the "requirement", the nutrient level where the response was maximised.

The ascending portion of the response is sometimes considered to be curved in nature with a smooth transition to the plateau. Almquist (1953) concluded from several experiments that plotting the log of nutrient intake in the suboptimal region usually results in a straight line response. The intersection of the ascending logdose line and the plateau gives an estimate of the "requirement". In the 1950s, plotting data on log-lined graph paper was an effective way to discover the parameters for non-linear regression. Since the 1980s it has been more efficient to fit polynomial or other curves with statistical software on computer. The Broken-Line Quadratic Model (BLQ) has a second order polynomial for the ascending region instead of the straight line (first order polynomial) in the BLL Model.

Fisher et al. (1973) presented a non-linear model that fitted a sigmoid response to amino acid intake for laying hens, the "Reading Model". Another sigmoid model, the saturation kinetics model, was shown to fit data from a wide variety of nutritional responses for different species by Morgan et al. (1975). These sigmoid models have two important differences from Almquist's (1953) log-intake model: first, the ascending portion may be sigmoid instead of quadratic; second, the maximum response is never attained, but only asymptotically approached. Therefore, with the sigmoid models, there is no clear concept of the "requirement" for maximum performance. Since the maximum response is only approached and never attained, there is no required amount of nutrient that yields the maximum response.

The model of Fisher *et al.* (1973) assumed that individuals follow the broken-line linear model with different values for the upper and lower plateaus. Curnow (1973) demonstrated that the response curve of a population of such individuals would then have a smooth response curve: "The slope increases from 0 to a maximum,  $<\beta$ , and then back to 0 as X varies from  $-\infty$  to  $+\infty$ " ( $\beta$ = value of the lower plateau). With example

**Table 1.** Data used in the analyses - The responses of broiler chicks to dietary protein and lysine concentrations from 9 to 18 d of  $age^{1}$ .

Trt <sup>2</sup>	Protein (g/kg)	Ly	vsine	Body weight gain	Feed conversion	
		(g/kg diet)	(g/kg protein)	g/9 d Mean ± SE	g feed/g gain Mean±SE	
1 (42)	170	5.0	29.4	$127.6 \pm 8.0$	$2.65 \pm 0.12$	
2	170	6.0	35.3	$192.6 \pm 12.8$	$2 \cdot 26 \pm 0 \cdot 07$	
3	170	7.0	41.2	$205.8 \pm 10.1$	$2.06 \pm 0.06$	
4	170	8.0	47.1	$228.6 \pm 4.0$	$1.99 \pm 0.06$	
5	170	9.0	52.9	$244.8 \pm 10.5$	$1.86 \pm 0.05$	
6	170	10.0	58.8	$219.5 \pm 12.1$	$2.06 \pm 0.11$	
7	170	11.0	64.7	$234.7 \pm 0.8$	$1.98 \pm 0.01$	
8	170	12.0	70.6	$241.7 \pm 13.2$	$1.85 \pm 0.09$	
9	230	7.0	30.4	$187.9 \pm 7.4$	$2.05 \pm 0.02$	
10	230	8.0	34.8	$222.2 \pm 5.7$	$1.87 \pm 0.04$	
11	230	9.0	39.1	$270.2 \pm 14.9$	$1.67 \pm 0.07$	
12	230	10.0	43.5	$295.3 \pm 5.6$	$1.57 \pm 0.05$	
13	230	11.0	47.8	$284.6 \pm 12.1$	$1.58 \pm 0.03$	
14	230	12.0	$52 \cdot 2$	$292.3 \pm 7.1$	$1.50 \pm 0.03$	
15	230	13.0	56.5	$310.8 \pm 23.2$	$1.48 \pm 0.06$	
16	230	14.0	60.9	$305.4 \pm 20.8$	$1.50 \pm 0.07$	

<sup>1</sup>Means ± SE of three pens (6 birds each at 7 d) per treatment.

<sup>2</sup>Treatment number.

data from an experiment on the egg production response to methionine intake, Curnow (1973) pointed out: "In a simple economic model, the optimum level of methionine would be the point at which the slope of the response curve equaled the ratio of the cost on an extra unit of methionine to the return from an extra unit of egg production".

Animal feeding trials do not always result in information in the very low (sigmoid), portion of the response curve (Robbins *et al.*, 1979). Therefore, simpler models are usually adequate to describe nutritional response data realistically. Robbins *et al.* (1979) compared two non-linear asymptotic curve models with the broken-line model. They emphasised the problem with asymptotic curve models: "If a curve which approaches an asymptote is chosen, we confront the task of choosing a definition for the estimated 'requirement'.... We have arbitrarily chosen the dose at which the response reaches 0.95 times the total response.... Given this arbitrary choice, the procedure is entirely objective".

The task of choosing a definition for the estimated "requirement" was approached differently by Almquist (1952, 1954a). The "Law of Diminishing Returns" was applied to feeding trial response data by Almquist who had moved from a university setting to a commercial company. The law of diminishing returns had to be interpreted economically. The best feeding level for the nutrient in question is not some "requirement" for maximum performance. The best feeding level is the nutrient concentration that maximises profits, calculated from the cost of the inputs, shape of the response curve, and value of the outputs.

Although a "requirement" is static, the profit maximising concentration is dynamic, changing whenever the cost of the nutrient or value of meat or eggs changes.

Wilson (1977) compared several sigmoid growth curves relating growth to the age of broilers, quail, turkeys and ducks. He pointed out that "Any model of growth which is to be used to answer commercial questions must therefore allow for the food to be described in terms of its quality as well as quantity". The sigmoid curve for each combination of dietary ingredients (quality) should be considered to properly maximise profits. The exact models will not only be functions of diet quality, feed consumption and genetics, but also functions of the environmental conditions that the birds are kept in. Any factors that affect feed consumption and growth rate (such as genetics, temperature, humidity, pelleting and air flow velocity) will affect the response curves to the various nutrients, and the feeding levels that achieve maximum profitability.

The data used here to compare various methods of estimating nutritional responses and requirements were from a feeding trial conducted with Cobb x Cobb broiler chicks raised in battery brooders from 9 to 18d of age (Experiment 1901kw; Table 1, Figure 3). The diets were based on maize, maize gluten meal, poultry grease, maize starch and cellulose to have exactly the same amino acid profiles with 170 and 230 g/kg crude protein. Lysine concentrations were obtained by adding 1-lysine to the diets. There were three pens of 6 chicks for each combination of protein and lysine concentration.

## ESTIMATING REQUIREMENTS WITH MULTIPLE RANGE TESTS

Separation of means using multiple range tests, although misguided, assumes that a dietary requirement has been met at the lowest concentration of the nutrient that results in a response that is not significantly different from the maximum response (at some arbitrarily chosen level of significance). Multiple range tests for estimating nutritional requirements are usually based on one-way analysis of variance. The Least Squares Means (LSM) results were included in these comparisons since they are sometimes used to estimate nutrient requirements. LSM results are often presented as if they were from a multiple range test. However, the LSM procedure of SAS (1990) gives pair-wise comparisons of means using Student's t-test, and thus is not a multiple range test per se.

Determination of a nutritional requirement by comparison of adjacent means is equivalent to using a subset of the available data and discarding the rest. Any regression based on a plausible biological model of the expected nutrient response relationship over a broad range should be preferable. Selected nutrient concentrations in the experiment may not have been chosen in the proper range and a large proportion of the work done will have been wasted even under the best of circumstances.

Basing the concept of "requirement" on the lack of a statistically significant response to an increasing input means that the experimenter cares most about that part of the response curve where the statistical test is least sensitive, a problem pointed out more than 50 years ago (Almquist, 1954b). The low power of the test and the high likelihood of Type II error inevitably tend to underestimate the requirement, as pointed out by Shearer (2000), although the underestimate may not be as severe in poultry species, which have a long experimental history compared with many other animals. The sources and magnitudes of error in nutritional requirement experiments with poultry are better known and easier to control. This should allow experiments of appropriate power to be designed and conducted. Use of multiple-range tests also ignores advances in biological and statistical models that have occurred since the early years of experimentation in nutrition.

Multiple range tests, such as Scheffé's (1953) are regarded as more conservative, or cautious, than others. They do not declare means to be significantly different unless there are larger differences than with less cautious tests, like Duncan's (1955). The more cautious a multiple range test, the less likely it is to declare means significantly different from the one giving the

maximum (or minimum) response, and the lower the requirement estimate will be. The cautious, or conservative, approach to feed formulation and nutritional requirements is to provide higher concentrations of each nutrient to be sure the requirement is met and performance is maximised. Paradoxically, the choice of a more conservative multiple range test leads to a lower, less conservative requirement estimate.

The differences in requirement estimates between multiple range tests (Tables 2 and 3) are due to the more cautious nature of some tests. The differences in requirement for the different performance measurements (growth versus feed conversion, Table 2 vs. 3) are expected. The requirement for maximum growth is usually lower than for maximum feed utilisation. Only Scheffé's (1953) and the pair-wise comparison's of the Least Squares Means procedure showed differences in requirements for growth versus feed conversion ratio. This is likely due to the amount of replication in the data set and the wide intervals in lysine concentrations that were fed, as well as the inability of some tests to discriminate between means.

The multiple range test results shown in Tables 2 and 3 demonstrate the difficulty in using simple mean comparisons to estimate requirements. Results from the least Squares Means test seem inconsistent since they use variances from each pair of means. The other tests pool variances from all the data. It is questionable whether data from both protein concentrations should be used or should the data from each protein concentration be analysed separately? Researchers often pool such data assuming each mean should have the same population variance and only report a pooled standard for all the means. Separate regression models are usually predicted for each level of other variables, as in Figure 1.

#### Advantages of multiple range tests

Multiple range tests are easy to implement in software packages and seem relatively easy to understand: The bird needs a certain amount of nutrient to meet maximum response. There is no estimate of the precision of the requirement's estimate for authors to present that would suggest doubt or controversy in their conclusions. This is only an advantage for authors not wanting a quantitative measure of confidence in their results. Multiple range tests are familiar to most scientists working in applied fields. Recent published research in the global poultry science literature contain examples of papers using multiple range tests to separate means of growth responses to graded levels of dietary nutrients.

**Table 2.** A comparison of several multiple range tests and Least Squares Means for the body weight gain data in Table 1. Means without common superscripts are different at P < 0.05.

Treatment	Mean	n	Bonferroni <sup>1</sup>	Duncan <sup>2</sup>	REGW <sup>3</sup>	Scheffé <sup>4</sup>	SNK <sup>5</sup>	Tukey <sup>6</sup>	Waller <sup>7</sup>	$LSM^8$
15	310.8	3	A mon	Angonia	A	A	A	A	A	AB
16	305.4	3	A	A	A	AB	A	A	AB	AB
12	295.3	3	AB	AB	A	ABC	A	AB	ABC	BC
14	292.4	3	AB	AB	A	ABCD	A	ABC	ABC	ABC
13	284.6	3	ABC	AB	AB	ABCD	A	ABCD	BC	ABC
11	270.2	3	ABCD	BC	ABC	ABCDE	AB	ABCDE	CD	D FG
5	244.8	3	BCDE	CD	BCD	ABCDEF	BC	BCDEF	CD	DEFG
8	241.7	3	BCDEF	CD	BCD	ABCDEF	BC	CDEFG	E	DEF
7	234.7	3	CDEF	DE	CDE	BCDEF	BC	DEFGH	E	DEFG
4	228.6	3	DEF	DE	CDE	CDEF	CD	EFGH	EF	DEFG
10	222.2	3	DEF	DEF	DE	CDEF	CD	EFGH	EF	DEFGH
6	219.5	3	DEF	DEF	DE best	DEF	CD	EFGH	EF	DEFGHI
3	205.8	3	EF	EFG	DE stir	EF	CD	FGH	FG	GHIJ
2	192.6	3	EF	FG	E	FG	D	GH	G	G IJ
9	188.0	3	F	G	E	FG	D	Н	G	HIJ
1	127.6	3	G	Н	F	G	E	test. How	Н	K
Requirement	$230\mathrm{g/kg}$	>	8g/kg	9g/kg	8g/kg	8g/kg	8g/kg	8 g/kg	9 g/kg	9 g/kg
rawings on a	Protein	<	9 g/kg	$10\mathrm{g/kg}$	$9\mathrm{g/kg}$	$9\mathrm{g/kg}$	$9 \mathrm{g/kg}$	$9\mathrm{g/kg}$	$10\mathrm{g/kg}$	$10\mathrm{g/kg}$

<sup>&</sup>lt;sup>1</sup>Bonferroni (Miller, 1981).

**Table 3.** A comparison of several multiple range tests and Least Squares Means for feed conversion ratio data in Table 1. Means without common superscripts are different at P<0.05.

Treatment	Mean	n	Bonferroni <sup>1</sup>	Duncan <sup>2</sup>	$REGW^3$	Scheffé <sup>4</sup>	$\rm SNK^5$	Tukey <sup>6</sup>	Waller <sup>7</sup>	LSM <sup>8</sup>
15 demonstrate	2.65	3	A	A	A	A	A	A	A	LSM <sup>8</sup>
16	2.26	3	В	В	В	AB	В	В	В	A
12	2.06	3	BC	C	BC	BC	C	BC	C	В
14 5) 5150 52	2.06	3	BC	$\mathbf{C}$	BC	BC	C	BC	C	CD
13	2.05	3	BC	C	BC	BC	C	BC	C	CD
11	1.99	3	BC	CD	C	BCD	C	BC	CD	D
5	1.98	3	BC	CD	C	BCDE	C	C	CD	CD
8	1.87	3	CD	D	CD	<b>BCDEF</b>	CD	CD	D	CD
7 Densen 1977.	1.86	3	CDE	D	CD	<b>BCDEF</b>	CD	CDE	D	CD
4) 45 3000	1.85	3	CDE	D	CD	BCDEF	CD	CDE	D	D
10	1.67	3	DEF	E	DE	CDEF	DE	DEF	E	D
6	1.58	3	DEF	EF	E	DEF	E	EF	EF	EF
3	1.57	3	EF	EF	E	DF	E	F	EF	EF
2	1.50	3	F	EF	E	F	E	F	F	EF
9	1.50	3	F	EF	E	F	E	F	F	F
hi hamasiqi	1.48	3	F	F	E	F	E	F	F	F
Requirement	$230\mathrm{g/kg}$	>	8g/kg	$9\mathrm{g/kg}$	8g/kg	$7\mathrm{g/kg}$	8g/kg	$8\mathrm{g/kg}$	$10\mathrm{g/kg}$	F
	Protein	<	$9\mathrm{g/kg}$	$10\mathrm{g/kg}$	$9\mathrm{g/kg}$	$8\mathrm{g/kg}$	$9\mathrm{g/kg}$	$9\mathrm{g/kg}$	$11\mathrm{g/kg}$	8g/kg

<sup>&</sup>lt;sup>1</sup>Bonferroni (Miller, 1981).

#### Disadvantages of multiple range tests

With multiple range tests, the actual requirement can only be on or between two levels of the nutrient that were fed, and there is no way to tell precisely what it is (Figure 2). Scientists who are familiar with multiple range tests are often unfamiliar with the controversial history of such procedures and the large numbers of statisticians who have warned of the dangers of indiscriminate use. Many papers investigating nutritional

<sup>&</sup>lt;sup>2</sup>Duncan (1955).

<sup>&</sup>lt;sup>3</sup>REGW (Welsch, 1977).

<sup>&</sup>lt;sup>4</sup>Scheffé (1953).

<sup>&</sup>lt;sup>5</sup>SNK, Student-Newman-Keuls (Miller, 1981).

<sup>&</sup>lt;sup>6</sup>Tukey (Hayter, 1984).

<sup>&</sup>lt;sup>7</sup>Waller (Waller and Duncan, 1969).

<sup>&</sup>lt;sup>8</sup>LSM, Least Squares Means (SAS, 2005).

<sup>&</sup>lt;sup>2</sup>Duncan (1955).

<sup>&</sup>lt;sup>3</sup>REGW (Welsch, 1977).

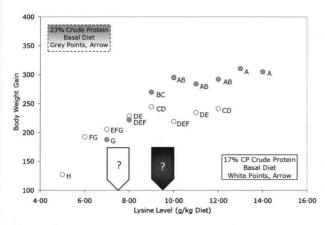
<sup>&</sup>lt;sup>4</sup>Scheffé (1953).

<sup>&</sup>lt;sup>5</sup>SNK, Student-Newman-Keuls (Miller, 1981).

<sup>&</sup>lt;sup>6</sup>Tukey (Hayter, 1984).

<sup>&</sup>lt;sup>7</sup>Waller (Waller and Duncan, 1969).

<sup>&</sup>lt;sup>8</sup>LSM, Least Squares Means (SAS, 2005).

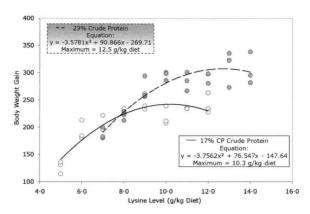


**Figure 2.** Data from an experiment to determine the lysine requirements of broiler chicks from 9 to 18d of age with the results of Duncan's New Multiple Range Test. The arrows indicate the best estimate of the "requirement".

requirements of poultry are based on experimental designs, often factorial, that employ graded levels of nutrients. Statisticians and concerned scientists have warned repeatedly against using multiple range tests in factorial arrangements or in experiments that could be analysed with some form of regression (Gill, 1973; Chew, 1976; Petersen, 1977; Little, 1978; Baker, 1980; Carmer and Walker, 1982; Dawkins, 1983; Nelson and Rawlings, 1983; Maindonald and Cox, 1984; Swallow, 1984; Gilligan, 1986; Perry, 1986; Cousens, 1988; Gates, 1991; Mihail and Niblack, 1991; Lowry, 1992; Pearce, 1993; Shearer, 2000). Many of the cited papers mention published examples where use of multiple comparisons either obscured the correct interpretation or led to a wrong interpretation. Results from multiple range tests are also dependent on the experimenter's arbitrary adoption of a level of significance.

## ESTIMATING REQUIREMENTS WITH SECOND ORDER POLYNOMIALS

Fitting polynomials to nutrient response data is based on the assumption that there is some level of input that results in maximum performance, the requirement. The GLM Procedure of SASv9·1 (2005) using the ordinary least squares method was used to fit the second order polynomial models. In the example data set, maximum body weight gains were reached at 10·3 and 12·5 g/kg dietary lysine, for the 170 and 230 g/kg protein diets, respectively (Figure 3). Nutritionists sometimes choose to set "requirements" at some input value other than 100% of the maximum response, as they may do with asymptotic models. Requirement estimates for 90, 95 and 99% of the maximum are included in



**Figure 3.** The second-order polynomial response model for the relationship between dietary lysine and broiler growth.

Table 7 for comparison to the asymptotic models.

Second-order polynomials describe relationships called "diminishing marginal productivity". The contributions to output from each successive unit of input are diminished until the maximum is reached. With a second order polynomial, adding units of input above the requirement is expected to actually decrease the response.

#### Advantages of second-order polynomials

The classical concept of "requirement" is clearly (if not accurately) defined as the nutrient concentration resulting in the maximum predicted response. Polynomial models are easy to fit to data (only three input levels are needed for quadratic responses, but three is not nearly enough points for the curve to be used to estimate the shape of the response a requirement with any confidence). Strictly speaking three points are enough to determine the coefficients of a quadratic. More points are required to determine whether the shape deviates from a quadratic and replication is required to indicate the precision of estimated coefficients. Maximum performance levels (or minimums for criteria such as feed conversion ratio), are easy to determine (by setting the first derivative equal to 0 and solving for x). Nutritional responses from low to high dietary concentrations exhibit an increase as deficiency is overcome, a plateau when the body is in a state of dynamic equilibrium and a decrease in performance when toxicity is reached. A second order, or quadratic, polynomial can fit the increase and decrease in performance well, but not the plateau. For instance, for the polynomial:

$$y = b_0 + b_1^* L + b_2^* L^2 (1)$$

where y is the dependent variable, L is the dietary lysine concentration, and  $b_0$ ,  $b_1$  and  $b_2$  are

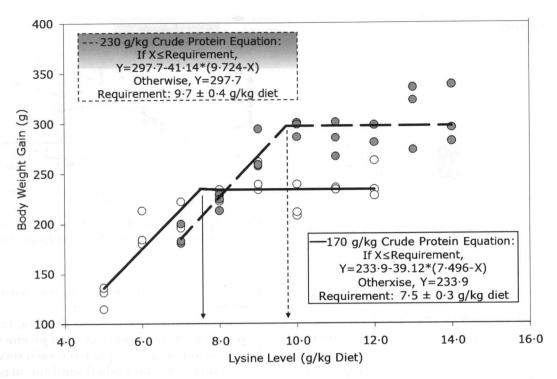


Figure 4. The broken-line with linear ascending portion model for the relationship between dietary lysine and broiler growth.

constants, the increase and decrease in response can be represented well if  $b_1$  is positive and  $b_2$  is negative.

#### Disadvantages of second-order polynomials

Most nutritional responses are believed to have a plateau or "safe" levels of inputs between the requirement for maximum response level and levels that are toxic. Second order polynomials are not able to characterise such data at all. The inclusion of data from inputs below or above those required for the maximum response have a larger effect on predictions near maximal responses than when using other models. That is, adding additional input levels at higher or lower levels changes the requirement estimate. Logically, adding extra input levels far from the should influence not requirement "requirement" (input at maximum response), but with this model it does.

# ESTIMATING REQUIREMENTS WITH BROKEN LINE OR SPLINE MODELS

The most commonly applied model to nutritional response experiments is the broken line model with the ascending line a linear function of dietary nutrient concentration (Figure 4). The broken line nutritional response models are a subset of spline models where the slope of one line is equal to 0. Broken line models assume that as concentrations of a nutrient are increased

in the diet, there is a change in response up to some point, the requirement, where the maximum (or minimum) response is reached. There is some plateau level above the requirement where the nutrient is neither helpful, nor toxic. The requirement is the x value of the point common to both lines.

$$y = b_0 + b_1^* x$$
, if  $x \le \text{requirement } y = \text{maximum}$  (or minimum), if  $x \ge \text{requirement}$ 

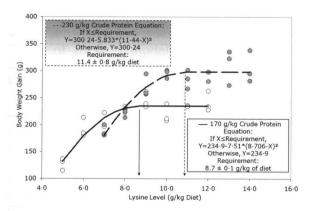
(2)

The broken line with ascending quadratic line model is similar and may fit some nutritional data better (Figure 5).

$$y = b_0 + b_1^* x + b_2^* x^2$$
,  
if  $x \le \text{requirement } y = \text{maximum}$  (3)  
(or minimum), if  $x \ge \text{requirement}$ 

The broken line with ascending quadratic line models diminishing marginal productivity until the level of the requirement is reached. After the level of the requirement, there are no further increases (or decreases) in response, marginal productivity is 0.

The NLIN Procedure of SASv9·1 (2005) using Marquardt's Method, was used to fit all non-linear models. The technique is iterative and requires good initial parameter estimates to give accurate requirement and other parameter estimates. The resulting parameter estimates include estimates of confidence in the requirement, maximum response, and rate constant (RC).



**Figure 5.** The broken-line with quadratic ascending portion model for the relationship between dietary lysine and broiler growth.

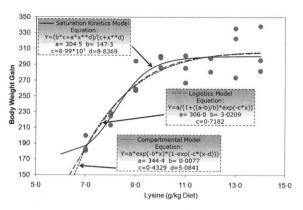
#### Advantages of broken-line models

The broken line models more closely represent theoretical ideas of the nature of nutritional responses than multiple range tests or polynomial models. The classical concept "requirement" is clearly defined as the nutrient input level resulting in maximum response for variables like growth, or a minimum for variables like carcase fat. The standard error of the requirement estimate is a measure of how good a particular experiment is. A small standard error of the requirement indicates that replication was adequate, but a large standard error of the requirement indicates that replication was not adequate considering the inherent variation in response.

#### Disadvantages of broken-line models

More levels of nutrient input are needed to get good estimates of the ascending and maximum response segments than with a quadratic polynomial. Broken line and other non-linear models are more difficult to fit than simple polynomials. The iterative techniques for fitting non-linear models do not always find the global minimum sum of squares and sometimes fail altogether to converge on a minimum. On the other hand, ordinary least squares methods fit polynomials to data very well as long as there are enough points.

The broken line with ascending quadratic portion is usually harder to fit to data than the broken line with ascending linear portion model, and the standard error of the requirement is usually larger for the broken line with ascending quadratic portion. In contrast to the quadratic polynomial, the broken line models fit the ascending and plateau portions well but have no feature or parameter to fit any decreasing (or toxic level) responses. There is no feature of the models for distinguishing concentrations of the nutrient that are toxic; some method should be



**Figure 6.** Three non-linear models for the relationship between dietary lysine and broiler growth of chicks fed 230 g/kg protein.

employed to eliminate higher nutrient concentrations that may be causing sub-maximal responses, so that those levels can be eliminated from the analyses, or more appropriate models can be found.

## ESTIMATING REQUIREMENTS WITH OTHER NON-LINEAR MODELS

Excellent reviews on the applicability of non-linear curves to animal growth and nutritional response curves have been written by Almquist (1953), Curnow (1973), Wilson (1977), Robbins et al. (1979), Fisher (1980), Mercer et al. (1978), Mercer (1982, 1992) and Gahl et al. (1991). A very large number of non-linear models have been fitted to nutritional response data. The three models compared here, the saturation kinetics model, a compartmental and a logistics model, are typical of the various non-linear models that may be used (Figure 6):

Compartmental model:

$$y = a^* e^{(-b^*x)*} (1 - e^{(-c^*(x-d))})$$
(4)

Logistic model:

$$y = a/(1 + ((a-b)/b)^* e^{(-c^*x)})$$
 (5)

where y is the response (growth, feed efficiency, etc.) a to c are constants, x is the dietary nutrient concentration and e is the base of natural logarithms,

Saturation kinetics model:

$$y = \frac{(b^*c + a^*X^d)}{(c + X^d)} \tag{6}$$

where y is the response (growth, feed efficiency, etc.), b is the intercept, a is the theoretical maximum, c is the nutrient rate constant, and d is the kinetic order of the response when x = 0. Many such models fit nutritional response data

**Table 4.** A comparison of several models' ability to fit nutritional response data using the Coefficient of Determination  $(R^2, Equation 8)$  and sums of the residuals squared (Resid. = Observed-Predicted (Py), Equation 9) for birds receiving 230 g/kg protein.

Model	All observations		Sum residuals squared				
			All observations	Transition 1	Transition 2		
	$R^2$	Adjusted $R^2$					
Second-order polynomial	0.8172	0.7898	8063	4068	3796		
Linear broken line	0.8349	0.8274	7281	2697	2432		
Quadratic broken line	0.8303	0.8141	7487	2975	2504		
Saturation kinetics	0.8384	0.8142	7129	2846	2541		
Compartmental	0.8357	0.8110	7249	3222	2870		
Logistic	0.8376	0.8222	m 1 20	3100	2785		

All observations = Lysine values: 7, 8, 9, 10, 11, 12, 13, and 14 g/kg.

Transition 1 = Lysine values: 7, 8, 9, 10, 11, and 12 g/kg.

Transition 2 = Lysine values: 9, 10, 11, and 12 g/kg.

well and it is difficult to choose a model that fits all nutritional response data better than others. The best rationalisation may be made for using the saturation kinetics model.

The saturation kinetics model is a superset of the Michaelis-Menten model for describing the velocity of enzyme-catalysed reactions (Michaelis and Menten, 1913).

$$v = \frac{(V_{\rm m}^*[S])}{(K_{\rm m} + [S])} \tag{7}$$

where v is the velocity of the reaction,  $V_{\rm m}$  is the theoretical maximum velocity, [S] is the concentration of the substrate,  $K_{\rm m} = [S]$  at half the maximum velocity.

In nutritional response experiments it may be theorised that one such enzyme-catalysed reaction (involving the nutrient in question) is limiting the growth and performance of each animal or bird. Nutritional responses should therefore follow Michaelis-Menten kinetics. Michaelis-Menten kinetics do not allow for an intercept different from 0, or for the kinetic order of the response to be different from 1; the saturation kinetics model has added features to include an intercept and different kinetic orders. Many, but certainly not all, non-linear models are asymptotic, approaching a maximum response, but never reaching it, and the concept of nutrients being toxic at high concentrations is not part of the models.

#### Accuracy of fitting non-linear models

The NLIN Procedure of SASv9·1 (2005) using Marquardt's Method, was used to fit all non-linear models. The technique is iterative and requires good initial parameter estimates to give accurate requirement and other parameter estimates. The resulting parameter estimates include estimates of confidence in the requirement, maximum response, and rate constant (RC).

#### Advantages of other non-linear models

The non-linear models more accurately depict biological responses than models that force responses to conform to straight lines.

#### Disadvantages of other non-linear models

The concept of "requirements" is not defined at all. More levels of nutrient input are needed to get good estimates of the ascending segment and maximum response than with polynomials. Nonlinear models are more difficult to fit than simple polynomials. There is no feature of the models for distinguishing concentrations of the nutrient that are toxic; some method should be employed to eliminate higher nutrient concentrations that may be causing sub-maximal responses so that those levels can be eliminated from the analyses.

#### STATISTICAL COMPARISON OF NUTRITIONAL RESPONSE MODELS

There is no simple answer to the question, "Which model is best for estimating nutritional requirements?" These regression models can be compared by considering the coefficient of determination statistics  $(R^2)$ . For a given model this statistic is:

$$R^{2} = 1 - \frac{\sum (\hat{Y}_{i} - \overline{Y})^{2}}{\sum (Y_{i} - \overline{Y})^{2}}$$
(8)

where  $Y_i$  is the *i*th observed value,  $\hat{Y}_i$  is the *i*th predicted value  $i=1,\ldots,n$  and  $\overline{Y}$  is the mean of the observations. It can be shown that  $R^2$  lies between 0 and 1, with higher values of  $R^2$  representing better fits. The data value of  $R^2$  is shown in column 2 in Table 4, for the respective models. The *F*-tests (see, e.g., Draper and Smith, 1966, Equation 2·6·11, pp. 62–64) for all models give P < 0.0001, hence we conclude that each model is a statistically significant model.

Based on these results, it is clear that the second-order polynomial model is the least well fitting of these 6 models, and the Saturation Kinetics model is the best both when all observations are compared and when only the transition set are compared. When considering the whole set of observations, the logistic and compartmental models are also very good, followed by the two broken-line models.

It is possible to make an adjustment to the  $R^2$  values in Table 4 to account for differences in the number of parameters in the models by dividing the numerator in the ratio part of [8] by (n-p) and dividing the denominator by (n-1), where n is the number of observations and p is the number of parameters. When this is done the second-order polynomial is still clearly the least adequate fit to this data. The superiority of the other models is therefore not due solely to their larger number of parameters.

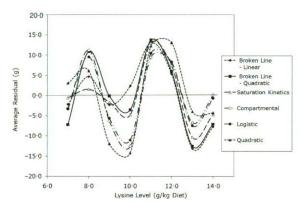
Another way to compare the six models is to look at the overall sum of squared residuals. In each case:

$$R_{\rm m} = \sum_{i=1}^{n} (O_i - E_i)^2 \tag{9}$$

All the models have residuals, the observed minus the predicted values, except the multiple range tests. The model with the lowest sum of squared residuals is the best overall fit to the data. The sum of squared residuals for all observations are displayed in column 4 of Table 4. As for the  $R^2$  comparisons, the second-order polynomial model separates itself out as having the worst fit of these models, since it has the largest sum of squared residuals value. The sum of squared residuals is a linear function of the  $R^2$  values, so the order of fit is exactly the same. In reality, for these data there is not much to distinguish these models from each other (apart from the second-order polynomial model).

These comparisons look quite appropriately at all the data across all lysine concentrations. However, given the importance of the region around the transition from ascending to plateau portion of the response, it is interesting to compare these fits for the middle (Transition Model 1) or middle 4 (Transition Model 2) lysine concentrations that surround this critical transition (Table 4). The plot of the residuals against dietary lysine concentrations (Figure 7) illustrates that the two broken-line and saturation kinetics models have the smallest residuals at the most important lysine concentrations (where the response is in transition from ascending to plateau phases).

For all lysine concentrations and the Transition Models 1 and 2, the sum of squared residuals obtained from Equation (9) are shown



**Figure 7.** Comparison of the average residuals for six non-linear models for the relationship between dietary lysine and broiler growth.

in columns 4, 5 and 6 of Table 4. An immediate observation is the big reduction in the sum of squared residuals for the respective models. In fact most of this is due to the absence of the values of the two highest lysine levels. It is clear (see Figure 7) that the observed values for these two cases have high variances, and they account for more than half of the sum of squared residuals of column 4 of Table 5. In that sense the comparisons are distorted.

For both the coefficient of determination and sum of squared residuals criteria, the linear broken line and quadratic broken line models provide the best fit to the Transition Model 1 data. For Transition Model 2, the linear broken line and saturation kinetics models provide the best fits, emphasising how important choosing nutrient input levels may be to estimating the best fit models and nutritional "requirements". Again, the second-order polynomial model gives the worst fit by a large margin compared to the other five models.

There is, for all cases, no model that can be declared clearly superior to the others from a statistical perspective. Each experiment should be evaluated based on the observed results and which model best fits the particular observed data, and the researchers goals in designing the experiment.

#### ECONOMIC INTERPRETATIONS OF NUTRITIONAL RESPONSE DATA

From the economic perspective, the choice of the optimal feeding level can be cast as the solution of a profit-maximisation problem. Where max  $\pi$  represents the maximum profit obtainable. In the most general form, such a problem can be stated as

$$\max \pi(x) = p \bullet y - w \bullet x - c \quad s \cdot t \cdot y = f(x) \quad (10)$$

**Table 5.** Economic interpretation of nutritional response data. It was assumed that the basal feed containing 7 g/kg lysine costs £0·14/kg, L-lysine costs £1·55/kg, and broilers were valued at £1·00/kg.

Dietary	y lysine	Feed intake	Fee	ed cost	Body weight gain	Revenue	Profit	Marginal profit	
g/kg		g/bird	£/1000 kg	£/1000 birds	g/bird	£/1000 birds	£/1000 birds	£/1000 birds	
7.0		384-34	140.00	53.81	187-36	187-36	133.55		
7.2		386-22	140.31	54.19	192-23	192.23	138-04	4.49	
7.4		389.71	140.62	54.80	198-36	198-36	143.56	5.52	
7.6		395-61	140.93	55.75	205.75	205.75	150-00	6.44	
7.8		404.43	141.24	57.12	214-29	214.29	157-17	7.17	
8.0		415.52	141.55	58.82	223-69	223.69	164.87	7.70	
8.2		426.95	141.86	60.57	233-53	233.53	172.96	8.09	
8.4		436.64	$142 \cdot 17$	62.08	243-33	243-33	181-25	8.29	
8-6		443.64	142.48	63-21	252.64	252.64	189-43	8-18	
8-8		448-17	142.79	63.99	261-12	261-12	197-12	7.69	
9.0		450.90	143.10	64.52	268-56	268-56	204.03	6.91	
9.2		452.50	143.41	64.89	274.88	274.88	209.99	5.96	
9.4		453.42	143.72	65.17	280-13	280.13	214.97	4.98	
9.6		453.95	144.03	65.38	284-41	284-41	219-03	4.06	
9.8		454.26	144.34	65.57	287-85	287-85	222-28	3.25	
10.0		454.43	144.65	65.73	290.58	290.58	224.84	2.56	
10.2		454.54	144.96	65.89	292.73	292.73	226.84	2.00	
10.4		454.60	145.27	66.04	294.43	294.43	228.39	1.55	
10.6		454.63	145.58	66-19	295.76	295.76	229.58	1.19	
10.8		454.66	145.89	66.33	296-81	296.81	230.48	0.90	
11.0		454.67	146.20	66.47	297.63	297.63	231.16	0.68	
11.2		454.68	146.51	66-61	298-27	298-27	231-66	0.50	
11.4		454.68	146.82	66.76	298.78	298.78	232.02	0.37	
11.6		454.68	147-13	66-90	299.18	299.18	232-28	0.26	
11.8		454.69	147.44	67.04	299.50	299.50	232-46	0.18	
12.0		454-69	147.75	67.18	299.75	299.75	232.57	0.11	
12.2		454.69	148.06	67.32	299.95	299.95	232-63	0.06	
12.4		454.69	148.37	67.46	300.11	300.11	232.65	0.02	
12.6		454.69	148-68	67-60	300-24	300-24	232.63	-0.01	
12.8		454.69	148.99	67.74	300.34	300.34	232.60	-0.04	
13.0		454.69	149-30	67.89	300-42	300.42	232.54	-0.06	
13.2		454.69	149-61	68-03	300-49	300.49	232.46	-0.07	
13.4		454-69	149-92	68-17	300.55	300.55	232.38	-0.09	
13.6		454.69	150.23	68-31	300-59	300.59	232.28	-0.10	
13.8		454.69	150.54	68-45	300-63	300-63	232.18	-0.10	
14.0		454.69	150.85	68-59	300-66	300-66	232.07	-0.11	

where y is the level of output, x is the input required to produce y, p is the price of output, w is the price of input,  $f(\bullet)$  is the input transformation (or production) function, and c are other costs associated with producing output y (Mas-Colell et al., 1995; Varian, 1992) solution to the problem requires taking the first derivative of the profit function. The optimal feeding level is then found by equating the derivative to 0.

In general, the price of output p and production cost c may depend on the level of produced output, while the cost of input may depend on the quantity of the purchased input. However, under certain circumstances, these variables can be assumed constant. For instance, if chicks are produced under contract, the perunit price of output may be fixed in advance regardless of the quantity produced. In the same way, the per-unit price of input can be fixed either by a contract or through advanced purchase. If this is the case, the solution to the profit maximisation problem simplifies somewhat, and

the optimal feeding level can be found from the condition

$$Pf'(x) = w, (11)$$

which can be interpreted as marginal revenue from an additional unit of input used is equal to the marginal cost of that additional unit. If the amount of input is constrained between  $x_{\min}$  and  $x_{\max}$ , then condition (11) is slightly modified to

$$pf'(x) - w = 0$$
 if  $x_{\min} < x < x_{\max}$ ,  
 $pf'(x) - w < 0$  if  $x = x_{\min}$   
 $pf'(x) - w > 0$  if  $x = x_{\max}$  (12)

If the broken-line linear model is used, the economic interpretation of the data is very simple. Below the maximum, the derivative of the production function f(x) is equal to the constant  $b_1$  (Equation (3)), i.e. each additional unit of input results in the same additional output, up to the maximum. When the maximum level of output is reached, there is no further

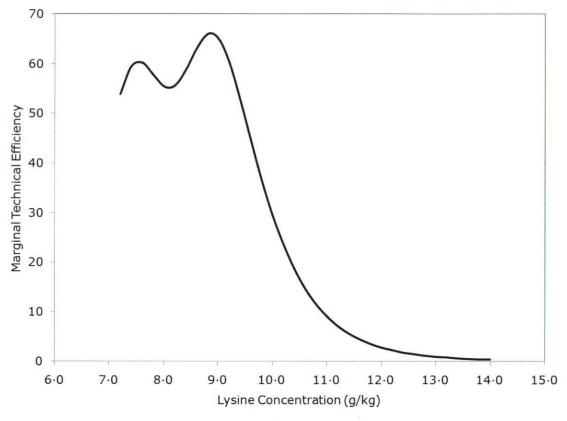


Figure 8. The marginal technical efficiency ( $\Delta$  Body Weight Gain/ $\Delta$  Lysine Intake) of dietary lysine as expected from the saturation kinetics model in Figure 6.

increase in output in response to additional units of input, i.e. f'(x) = 0. Thus, unless the marginal revenue  $pb_1$  is exactly equal to the marginal cost of input w, it must be cost effective to either feed at the basal level  $x_{\min}$  or at the requirement level  $x_{\max}$ , never at an intermediate level.

If the broken line with ascending quadratic model is used, then the nutritionist can use either: (1) a profit maximisation approach to find the most economical feeding level, or (2) feed at the break point, or "requirement".

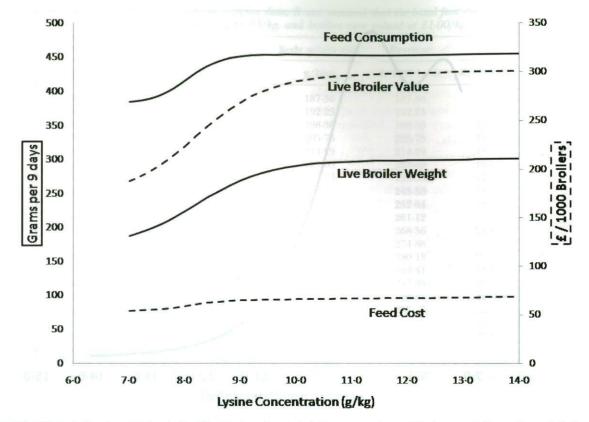
With response models such as the saturation kinetics, Reading or exponential, there is no technical "requirement" level for feeding. However, the profit maximisation approach can still be applied to decide on the most economical concentration of each nutrient to feed. Equal amounts of additional input now result in different amounts of additional output, i.e. the derivative f(x) of the production function is no longer constant. Typically, the marginal revenue pf'(x) is greater than the marginal cost w at lower levels of input, i.e. marginal profit is positive. However, the additional output zproduced in response to each additional unit of input decreases overall concentrations increase.1 The technical relationship between dietary lysine concentration and the

marginal efficiency of lysine utilisation ( $\Delta$  body weight gain/ $\Delta$  lysine consumption; Figure 8) illustrates that, as lysine concentration increases, the marginal efficiency approaches 0. From the economic perspective this implies that the marginal revenue decreases and approaches marginal cost, i.e. the marginal profit approaches 0.

For the example in Table 5, the body weight and feed consumption data for the chick's response to lysine concentration have been predicted by the saturation kinetics model (Figure 9). It was assumed that the basal feed containing 7 g/kg lysine costs £0·14/kg, L-lysine costs £1·55/kg, and broilers were valued at £1·00/kg.

When any model is applied, the point of maximum profits can be predicted from the technical (response equation) relationship and the costs and values of the inputs and outputs (Table 5). In this example, 12.4 g/kg lysine maximised profit over quantities of input. The marginal profit column was included in Table 5 to show how the contributions to profit diminish as lysine is added to the diet, and illustrate that profits are maximised when marginal profit become equal to 0.

Mathematically speaking, this requires that the production function f(x) is concave, i.e. its second derivative f''(x) is negative. This condition is satisfied for the broken line linear and quadratic ascending segments non-linear models for sufficiently high levels of inputs X.



**Figure 9.** Technical and economic relationships between dietary lysine concentration and feed consumption and growth (left vertical axis) and the cost of growth and value of growth (right vertical axis). The **saturation kinetics** model parameters for growth are from Figure 6, and for feed consumption are a = 454.7, b = 382.5, c = 0.004 and d = 26.02.

The basic shape of the marginal revenues curve (Figure 10) is the same as the marginal technical efficiency curve (Figure 8). Maximum profits are achieved when marginal costs and marginal revenues are equal, i.e. the curves intersect. If marginal cost is less than the marginal revenue, additional profit can be generated by increasing input level. On the other hand, if marginal costs are greater than marginal revenues, then money is lost unnecessarily.

Note that the economic model discussed above is very simple and in practice models should include more than feed costs. However, the basic principles illustrated here also apply to more complex models that include inputs such as housing and labour costs. Practical models should be based on producing a bird of a certain market weight, not age as in the example experiment.

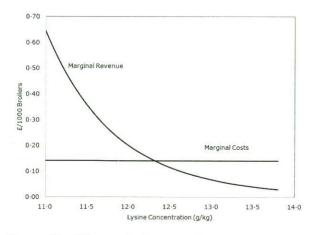
Note also that 12.4g/kg should not be interpreted as the lysine "requirement". If the cost of lysine or value of broilers were to change, the concentration of lysine to maximise profits would also change (Table 6). Consistently with economic theory, all things being equal, if the value of the output (broilers) increases, then the level of production should increase, and the amount of lysine fed should increase. But if the

cost of the input (lysine) increases, then the use of the input should decrease and the production level decreases as well.

# HOW SHOULD THE NUTRIENTS (INDEPENDENT VARIABLES) BE EXPRESSED?

It is most difficult to compile technical and economic models with a large number of meaningful inputs. A common simplification that has been made is to express the amino acid requirements in relation to one amino acid or all amino acids (dietary crude protein or nitrogen  $\times$  6.25). Almquist (1952), Boorman and Burgess (1986) and Baker and Han (1994) have written excellent reviews on this subject.

If the dietary lysine content is expressed as the concentration relative to the dietary protein concentration instead of relative to the diet, then the lysine by protein interaction is no longer significantly different from 0 (Figure 11). Neither is there a difference in lysine requirements when different protein concentrations are fed. The formulating nutritionist must decide on the best way to express nutritional requirements as well as the important nutrients to model from an economic perspective to truly maximise profits.



**Figure 10.** The marginal costs and revenue from feeding several concentrations of lysine to broiler chicks. The data were predicted by the saturation kinetics models of Figure 6.

For commercial applications, more complex models with multiple nutritional inputs may prove advantageous. Performance may be improved by adding one amino acid to a diet, or by adding a mixture of amino acids from practical feed ingredients (protein). A model depicting the substitution of amino acids from crystalline or intact protein sources demonstrated the value of considering multiple factors when attempting to maximise profits (Sterling et al., 2005).

#### THE REQUIREMENT DILEMMA

The value of any requirement or response estimate depends on a number of factors: the inherent experimental variation from genetic and environmental sources, the amount of replication and experimental design factors such as the concentrations of the nutrient in question. There may also be complicating factors in a factorial arrangement in experiments, such as protein concentrations, exogenous enzyme supplementation or different bird genotypes. Statistical models should facilitate understanding the answers to questions on appropriate feeding levels.

The choice of a statistical model is critical to interpreting nutritional requirement experiments (Table 7). The example data (Table 1) were not chosen because the different interpretations were particularly exaggerated, yet large differences in lysine "requirements" were observed: The use of a "cautious" or "conservative" multiple-range test could justify feeding no more than  $9\,\mathrm{g/kg}$  lysine to maximise growth, compared to  $10.9\pm0.2$ , 12.8 and  $13.2\,\mathrm{g/kg}$  for the ascending quadratic with plateau, logistics and saturation kinetics models,

**Table 6.** The influence of feed cost and broiler value on the lysine concentrations (g/kg diet) that maximise profits using the saturation kinetics models of Figures 6 and 9 with 230g/kg crude protein.

Lysine (£/kg)	Broiler value (£/kg)				
	0.40	0.56	0.70		
0.6	10.4	10.6	10.8		
1.2	9.8	10.0	10.4		
1.8	9.4	9.8	10.0		
2.4	9.0	9.4	9.8		

respectively (with inexpensive lysine and high broiler values).

Nutritionists intuitively know (assume) that there is some concentration of each nutrient that maximises performance (for each genetic stock under each set of each set of environmental conditions). They also know intuitively that there is some concentration of each nutrient that will result in toxicity and decrease performance. Orthogonal contrasts and multiple range tests seem appropriate for asking the question "Will an additional unit of nutrient significantly improve performance (given the constraints of what is known about the variation in ingredients and accuracy of weighing ingredients for feed mixing)?" The broken-line models seem appropriate for answering the question: "What is the nutritional requirement for maximum performance?" And the models with ascending curves seem appropriate for answering the question: "What is the shape of the response curve to graded concentrations of a nutrient (up to the point when it becomes 'safe' or toxic?'

The broken-line models have the advantages of fitting the data well and having a clear definition of what the requirement is. The broken-line with ascending quadratic model would seem to be a good compromise for most responses. It clearly defines the requirement. It depicts diminishing marginal productivity as would be expected from the theory that nutritional responses should follow enzyme kinetics. It gives a cautious estimate of nutritional requirements, always higher than multiple range tests or the broken-line with ascending linear model. Unfortunately, in our experience it is the most difficult to fit statistically. In general, with contemporary computational power, statistical fitting is now much easier and not the limiting factor that it once was.

Biological systems rarely work in perfectly linear fashions, so the non-linear models are more intuitive for careful observers of scientific data than models with linear functions. If the interpreter believes that biological responses should approach the maximum response in an asymptotic fashion, then one of the other

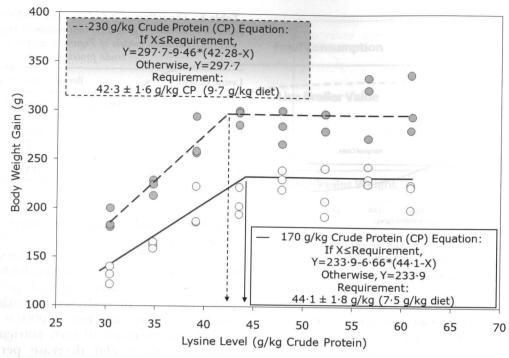


Figure 11. The broken-line with linear ascending portion model for the relationship between dietary lysine and broiler growth when lysine concentration is expressed as a function of dietary protein concentration.

**Table 7.** The influence of statistical method used on the lysine concentrations (g/kg of diet) that satisfy "nutritional requirements" for growth with 230g/kg crude protein.

Multiple Range Tests (between the highest level different from the maximum and the lowest level not different from the maximum, P < 0.05)

	or marginar revenues	(38)
	Bonferroni	A//// >8, <9 g/kg
	Duncan	$>9, <10 \mathrm{g/kg}$
	Ryan-Emot-Gabriel-Weisch	>8, <9 g/kg
	Scheffé	>8, <9 g/kg
	Student-Newman-Keuls	>8, <9 g/kg
	Tukey	>8, <9 g/kg
	Waller	>9, <10 g/kg
Edward Control	Least Squares Means	$>9, <10 \mathrm{g/kg}$
Quadratic polynomial		
	Maximum	$12.6\mathrm{g/kg}$
	0.99 times the maximum	$11.8\mathrm{g/kg}$
	0.95 times the maximum	$10.6\mathrm{g/kg}$
direction and a viscosition of	0.90 times the maximum	$9.8\mathrm{g/kg}$
Broken Line (Spline) Model		0 0
	Linear Ascending	$9.7 \pm 0.4 \mathrm{g/kg}$
-amplement redounts	Quadratic Ascending	$10.9 \pm 0.2 \mathrm{g/kg}$
Saturation Kinetics Model		
	0.99 times the asymptote	$11.0\mathrm{g/kg}$
	0.95 times the asymptote	$9.7\mathrm{g/kg}$
	0.90 times the asymptote	9.1 g/kg
	Lys = $£0.6$ /kg, Broilers = $£0.40$ /kg	$10.4\mathrm{g/kg}$
	Lys = $£0.6/kg$ , Broilers = $£0.7/kg$	$10.8\mathrm{g/kg}$
	Lys = £2.4/kg, Broilers = £0.40/kg	$9.0\mathrm{g/kg}$
	Lys = £2.4/kg, Broilers = £0.70/kg	$9.8\mathrm{g/kg}$
Logistics Model	Long Sorthern for the first	
	0.99 times the asymptote	$12.8\mathrm{g/kg}$
	0.95 times the asymptote	10.5 g/kg
Allthing hy way by	0.90 times the asymptote	$9.5\mathrm{g/kg}$
Compartmental Model		0, -0
	0.99 times the maximum	$12.5\mathrm{g/kg}$
	0.95 times the maximum	$10.6\mathrm{g/kg}$
	0.90 times the maximum	9.6 g/kg

non-linear, exponential models may be preferred. Regardless of the model chosen, the economic ramifications should be considered for practical feed formulation (Lerman and Bie, 1975).

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